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No significant adverse effects on rainbow trout of copper at 10.9 and 21.5 µg litre^{-1} were found in a similar 30-day exposure - A. hydrophila infection study. Mortality of Cu-exposed infected fish was not different from that in control-infected fish and haematocrits and leucocrits showed no significant differences related to copper concentration.

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THE RESPONSE OF RAINBOW TROUT *SALMO GAIRDNERI* TO *AEROMONAS HYDROPHILA* AFTER SUBLETHAL EXPOSURES TO PCB AND COPPER

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ABSTRACT

Rainbow trout were continuously exposed to sublethal PCB concentrations of 7·3 and 14·7 μg litre^{-1} *for 30 days and infected with* A. hydrophila *or sham-injected. Mortality of PCB-exposed infected fish was significantly lower than control-infected fish. Survivors of the infection at all exposure concentrations had elevated leucocrits and decreased haematocrits compared with their respective shams. PCB-exposed sham fish had significantly higher leucocrits at both concentrations and significantly lower haematocrits at 14·7 μg litre*$^{-1}$ *compared with control-shams. Among the infected fish, leucocrits at both PCB concentrations were higher than the control, but haematocrits were not different at any concentration.*

No significant adverse effects on rainbow trout of copper at 10·9 and 21·5 μg litre^{-1} *were found in a similar 30-day exposure–*A. hydrophila *infection study. Mortality of Cu-exposed infected fish was not different from that in control-infected fish and haematocrits and leucocrits showed no significant differences related to copper concentration.*

INTRODUCTION

In recent years, chemical pollutants have been increasingly implicated in infectious disease occurrences in fish (for reviews see Snieszko, 1974; Overstreet & Howse, 1977; Sindermann, 1979). Most of the evidence is circumstantial, coming either from field studies that showed increased frequencies of disease in fish populating heavily polluted areas, or from disease outbreaks, often of uncertain etiology, that occurred during laboratory toxicity studies.

Recent pollutant–disease interaction laboratory studies demonstrated altered susceptibility to pathogens and impaired immunological responses in fish exposed

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to chemical pollutants, mostly heavy metals. Zinc, copper and methylmercury exposures resulted in suppressed antibody response to bacterial and viral antigens in two exotic fish species (Sarot & Perlmutter, 1976; Roales & Perlmutter, 1977). Stevens (1977) reported that copper reduced survival to a natural *Vibrio anguillarum* challenge and reduced antibody titers in coho salmon *Oncorhynchus kisutch* following a simultaneous vaccination and chemical exposure. Hetrick *et al.* (1979) found that rainbow trout *Salmo gairdneri* were more susceptible to infectious haematopoietic necrosis (IHN) virus after copper exposure. Increased susceptibility of steelhead rainbow trout to *Yersinia ruckeri* due to copper was also reported by Knittel (1981). In coho salmon, Sugatt (1980) reported increased susceptibility and reduced agglutinin titers to *V. anguillarum* following chromium exposure.

The purpose of this study was to determine if extended exposure to sublethal concentrations of polychlorinated biphenyls (PCB) or to copper alters the susceptibility of rainbow trout to *Aeromonas hydrophila* infection. *A. hydrophila* is a gram negative bacterium considered ubiquitous in freshwater where it causes bacterial haemorrhagic septicemia (BHS) in many fish species (Bullock *et al.*, 1971; Hazen *et al.*, 1978). BHS has been strongly linked to environmental stress in fish (Wedemeyer, 1974; Richards & Roberts, 1978). This study attempted to detect physiological changes useful for interpreting the whole animal response by measuring haematocrits and leucocrits of survivors. The leucocrit is a rapid procedure similar to the microhaematocrit but for estimating total peripheral leucocytes and thrombocytes. It was developed by McLeay & Gordon (1977), who demonstrated its utility for detecting various acute stresses, including those from pulpmill effluents.

MATERIALS AND METHODS

Fish

Rainbow trout *Salmo gairdneri* for the main experiments were generously supplied by the US Fish and Wildlife Service, National Fisheries Research Laboratory, LaCrosse, WI, USA. French River Coldwater Hatchery (MN Department of Natural Resources) supplied the rainbow trout used for the solvent-effect experiment. The experiments were run in Lake Superior water which had been passed through a sandfilter, heated when necessary, filtered through two sets of 50 micron Cuno filters (AMF Inc., Meriden, CT, USA) and sterilised with ultraviolet light. Measured water quality characteristics were: total hardness, $43{\cdot}8 \pm 1{\cdot}5$ mg litre^{-1} as $CaCO_3$; alkalinity, $42{\cdot}0 \pm 1{\cdot}8$ mg litre^{-1} as $CaCO_3$; pH, 7·1–7·2 and dissolved oxygen, 6·8–8·4 mg litre^{-1}. Water temperatures varied from 15 to 18·5 °C over the experimental period. The trout were fed PR-9 trout food (Glenco Mills), maintained on a 16 h light:8 h dark photoperiod and acclimated at least 1 month prior to use. Separate nets, siphon tubes, scrubbers and sample containers

were used for each tank and disinfected between each use in a 200 mg litre^{-1} Roccal® II (National Laboratories, Montvale, NJ, USA) to minimise cross contamination.

Bacteria

A culture of *Aeromonas hydrophila* isolated from diseased brown trout *Salmo trutta* at the Genoa Fish Disease Laboratory, WI, USA (case No. 77–22) was used for the experimental infection. The bacterium was a motile, gram negative bacillus; positive for cytochrome oxidase, catalase, indole and butanediol dehydrogenase; oxidatively and fermentatively used glucose and gave presumptive identification as *A. hydrophila* with both Rimler–Shotts agar (Shotts & Rimler, 1973) and AH medium (Kaper *et al.*, 1979). The culture was maintained on Trypticase® Soy Agar (TSA, Becton Dickson & Co., Cockeysville, MD, USA) slants at 5°C and subcultured once a month. Prior to use, the bacteria were passed three times in rainbow trout in an attempt to enhance virulence factors that may have been attenuated by repeated artificial culture.

A. hydrophila was administered to the fish by intraperitoneal (IP) injection of 0·1 ml of a suspension prepared from a 24 h culture grown at 25°C on TSA, washed and suspended in 0·85% sterile saline. Bacterial numbers were estimated by the standard plate count and reported as CFU per millilitre (CFU = colony-forming units). Isolates from kidney samples were subcultured on TSA and selective media (Shotts & Rimler, 1973; Kaper *et al.*, 1979) and were morphologically and biochemically evaluated.

Analytical procedures

For the PCB experiment, stock solutions of Aroclor® 1254 of 5·726 mg ml^{-1} and 2·863 mg ml^{-1}, dissolved in dimethylformamide (DMF), were used to fill each toxicant syringe. Each syringe injected the same volume, 8·5–8·7 μl per cycle, so the DMF concentration added was 4·3–4·4 μl litre^{-1} at both PCB concentrations. Weekly water samples from each tank and effluent samples before and after activated carbon filtration were extracted in redistilled hexane by rapid stirring for 90 min. PCB concentrations were analysed on a Hewlett–Packard gas chromatograph with an electron-capture detector and calculated on a H–P Data System.

Mean PCB concentrations, $\pm$SD, were $<0{\cdot}2$ μg litre^{-1} for control ($N = 20$), $7{\cdot}3 \pm 1{\cdot}2$ μg litre^{-1} ($N = 18$) and $14{\cdot}7 \pm 2{\cdot}0$ μg litre^{-1} ($N = 18$) with no significant differences between replicate tanks ($p = 0{\cdot}05$). A 25-day LC_{50} for Aroclor 1254 in rainbow trout of 27 μg litre^{-1} has been reported by Mayer *et al.* (1977). Mean recovery of spiked samples was 106% (range 92–118%). Mean PCB removal by the activated carbon filter was 70%.

For the copper experiment, stock solutions of 4·81 mg ml^{-1} and 2·41 mg ml^{-1} were prepared by dissolving reagent grade $CuSO_4$ in deionised water acidified with concentrated HNO_3. Water samples collected weekly from each exposure tank were

acidified with HNO_3 and analysed for copper on a Perkin–Elmer atomic absorption spectrophotometer with a graphite furnace (Perkin–Elmer, 1977).

Mean copper concentrations were $2{\cdot}5 \pm 0{\cdot}66\,\mu g$ litre^{-1} ($N = 14$) for lake water control, $10{\cdot}9 \pm 2{\cdot}0\,\mu g$ litre^{-1} ($N = 20$) and $21{\cdot}5 \pm 4{\cdot}1\,\mu g$ litre^{-1} ($N = 20$), replicates combined. McKim *et al.* (1978) reported a maximum acceptable toxicant concentration to rainbow trout for copper (as $CuSO_4$) of $19\,\mu g$ litre^{-1} based on embryo–larval exposures in Lake Superior water.

Biological exposures

An intermittent-flow diluter with a separate injector and 50 ml gas-tight glass syringe for each toxicant concentration provided two litres each per cycle of control and two toxicant concentrations. A flow-splitter (Benoit & Puglisi, 1973) divided each concentration equally among four randomly assigned 30 cm × 91 cm × 30 cm glass aquaria, each holding 55 litres. The 90% volume-replacement time in each tank was about 7·5–8 h (Sprague, 1969).

Each experiment consisted of a 30-day chemical exposure followed by a challenge with *A. hydrophila*. Fish in two randomly chosen tanks at each exposure concentration were given an IP injection of *A. hydrophila*, and those in the other two were given a 0·1 ml IP injection of sterile 0·85% saline as negative controls on handling stress, injection trauma and delayed toxicity.

To initiate the PCB experiment, twenty rainbow trout, $5{\cdot}74 \pm 1{\cdot}72$ g and $8{\cdot}4 \pm 0{\cdot}8$ cm total length ($N = 240$), were added randomly to each test aquaria. After 30 days of exposure, the PCB addition was stopped, the fish were corralled in the front part of each tank with a temporary screen divider, removed individually, anaesthetised in 70 mg litre^{-1} of Finquel (tricaine–methane sulphonate), measured and weighed, given the appropriate disease treatment and returned to the tank behind the divider. Fish in the bacterial-challenged replicate tanks were inoculated with $1{\cdot}2 \times 10^8$ CFU per fish. Dead fish were collected as they were observed and examined for gross pathology. Samples of kidney tissue were asceptically inoculated onto TSA plates and incubated for 48 h at 25°C. To facilitate the sampling procedure, the experiment was terminated on two consecutive days, one replicate exposure-disease treatment per day, after a 14- or 15-day infection period. The tail of each survivor was amputated and blood was collected from the severed caudal vein into three narrow-bore heparinised microhaematocrit tubes ($0{\cdot}55 \pm 0{\cdot}05$ mm inside diameter, Sherwood Industries Ltd, St Louis, MO, USA) and leucocrits and haematocrits were determined by the procedure of McLeay & Gordon (1977). As recommended by these investigators, the sampling intervals were standardised: all tubes were centrifuged within 12 min of collection and measured within 1 h of centrifugation. In an attempt to determine sustained infections due to *A. hydrophila*, kidney tissue samples were inoculated onto TSA plates.

Because no DMF control was run during the PCB experiment, a separate experiment was run with similar-sized rainbow trout, 4·0–6·5 cm total length, to

determine if the DMF concentration added with the PCB affected haematocrits or leucocrits. Trout were exposed in duplicate tanks in lake water and lake water with 4·3–4·4 μl litre^{-1} DMF. Haematocrits and leucocrits were measured as previously described from randomly selected fish after 7, 14 and 34 days of exposure.

Because of their larger size (41·7 $\pm$ 13·2 g and 15·7 $\pm$ 1·8 cm, $N = 120$), only ten rainbow trout per tank could be used for the copper experiment. Procedures were those used during the PCB experiment except as follows: termination was 17 and 18 days after the bacterial challenge and blood was collected by caudal venipuncture using a heparin-rinsed disposable needle and syringe and microhaematocrit tubes filled from the syringe.

Statistical analysis

All data were analysed by Student's *t*-test for differences between replicate tanks. No significant differences were detected $p = 0{\cdot}05$); therefore, replicate data were combined. Growth data were analysed by one-way ANOVA (Steel & Torrie, 1960). Mortality data were analysed by the Mantel–Haenszel test (Mantel, 1966). Haematocrit and leucocrit data were analysed by two-way ANOVA using the BMDP2V program (Regents of the University of California, 1977) on a PDP-11/70 computer (Digital Equipment Corp., Maynard, MA, USA). Differences between treatment means were analysed by Duncan's new multiple range test (Steel & Torrie, 1960). In statistical computations involving haematocrits and leucocrits, each fish was represented by one value, the mean of the three replicate samples. However, in some cases this value represented the mean of two or, less frequently, one replicate because some replicates were lost due to breakage or unacceptable quality. To evaluate replicate sample variability of haematocrits and leucocrits, one value was randomly selected from each data subset (fish) with three measurements and the difference, $\bar{X}_1 - \bar{X}_3$, recorded. The process was repeated selecting two values and taking $\bar{X}_2 - \bar{X}_3$.

RESULTS

Thirty days of exposure to PCB at 7·3 and 14·7 μg litre^{-1} produced no detectable effects on survival and growth of rainbow trout ($p > 0{\cdot}05$). Except for one mortality at 7·3 μg litre^{-1}, all fish appeared healthy throughout the PCB-exposure period. The mean weight gain for all fish during this period was 62·5 %.

Results of the *A. hydrophila* infection are summarised in Table 1. PCB-exposed fish were significantly less susceptible to *A. hydrophila* than the non-exposed fish with 5·2 % and 0 % mortality at 7·3 and 14·7 μg litre^{-1}, respectively, compared with 32·4 % at the control. Differences in mortality between the two PCB concentrations were not significant. In addition to their lower total mortality, PCB-exposed fish

TABLE 1

MORTALITY AMONG RAINBOW TROUT INFECTED WITH *Aeromonas hydrophila* OR SHAM-INJECTED SUBSEQUENT TO A 30-DAY EXPOSURE TO CONTROL (LAKE WATER) AND TWO CONCENTRATIONS OF PCB (REPLICATES COMBINED)

Exposure concentration	*Disease treatment*[a]	*Number/total sample*[b]	*Mortality Percentage*	*Onset (h)*	*Cessation (h)*	*Proportion positive for* A. hydrophila
Control, <0·2 μg PCB litre^{-1}	Sham	0/40	0	—	—	—
Control	Infected	12/37	32·4	30	72	12/12
7·3 μg PCB litre^{-1}	Sham	0/40	0	—	—	—
7·3 μg PCB litre^{-1}	Infected	2/39*	5·2	48	120	2/2
14·7 μg PCB litre^{-1}	Sham	0/39	0	—	—	—
14·7 μg PCB litre^{-1}	Infected	0/39**	0	—	—	—

[a] Infected fish were given a 0·1 ml IP injection of an *A. hydrophila* saline suspension containing 1·2 × 10^9 CFU per millilitre. Sham-injected fish were given a 0·1 ml IP injection of 0·85% sterile saline.

[b] Total sample varies from initial 40 because some fish jumped from inadequately covered tanks and died 2 days after the start of the PCB exposure, except for one mortality at 7·3 μg litre^{-1}.

* Statistically different from control-infected (observed $\chi^2 = 7{\cdot}58$, degrees of freedom = 1, $0{\cdot}005 < p < 0{\cdot}01$).

** Statistically different from control-infected (observed $\chi^2 = 15{\cdot}44$, degrees of freedom = 1, $p < 0{\cdot}005$).

appeared slower in succumbing to the bacteria. Ten (of 12) control fish had died by the time the first exposed trout died. No saline sham-injected fish died at any concentration.

Kidney samples from all mortalities yielded numerous colonies confirmed biochemically as *A. hydrophila*. Gross necropsy of these fish showed some or all of the following pathological signs of BHS: petechiae at the bases of fins and around the vent, haemorrhages or petechiae on the intestines and other internal organs, purulent yellow to serosanguinous drainage from the intestine, enlarged spleen and distended air bladder. Kidney samples of only six fish from various exposure concentrations at termination yielded one or two colonies presumptively identified as *A. hydrophila*, suggesting no sustained infections.

Leucocrit and haematocrit values of surviving trout from the PCB experiment and statistical comparisons between experimental groups are summarised in Table 2. *A. hydrophila*-infected fish at all exposure levels had increased leucocrits and decreased haematocrits compared with their respective sham-injected exposure group. Among the sham-injected groups, PCB-exposed fish at both 7·3 μg litre^{-1} and 14·7 μg litre^{-1} had significantly higher leucocrits than control fish. Also among

TABLE 2

LEUCOCRIT AND HAEMATOCRIT VALUES OF RAINBOW TROUT SURVIVING A 30-DAY PCB EXPOSURE AND SUBSEQUENT *A. hydrophila* INFECTION OR SHAM INJECTION (REPLICATES COMBINED)

Exposure concentration	*Disease treatment*	*Leucocrit (%)*	*Haematocrit (%)*
Control, <0·2 μg PCB litre^{-1}	Sham	1·24 ± 0·23[a] (33)	34·9 ± 3·6 (40)
Control	Infected	1·55 ± 0·36 (20)	30·8 ± 3·9 (26)
7·3 μg PCB litre^{-1}	Sham	1·43 ± 0·23 (35)	33·5 ± 3·7 (40)
7·3 μg PCB litre^{-1}	Infected	1·73 ± 0·34 (22)	30·4 ± 3·1 (37)
14·7 μg PCB litre^{-1}	Sham	1·47 ± 0·25 (37)	32·1 ± 2·8 (39)
14·7 μg PCB litre^{-1}	Infected	1·77 ± 0·31 (34)	30·8 ± 2·3 (36)

[a] Mean ± standard deviation ( ) = number of fish.
Statistical significance of differences by Duncan's new multiple range test (** = $p < 0.01$, * = $p < 0.05$, ns = not significant).

Comparison	*Leucocrit*	*Haematocrit*
Control-sham versus Control-infected	**	**
7·3 μg PCB litre^{-1}-sham versus 7·3 μg PCB litre^{-1} infected	**	**
14·7 μg PCB litre^{-1}-sham versus 14·7 PCB litre^{-1}-infected	**	ns
Control-sham versus 7·3 μg PCB litre^{-1}-sham	**	ns
Control-sham versus 14·7 μg PCB litre^{-1}-sham	**	**
7·3 μg PCB litre^{-1}-sham versus 14·7 μg PCB litre^{-1}-sham	ns	ns
Control-infected versus 7·3 μg PCB litre^{-1}-infected	*	ns
Control-infected versus 14·7 μg PCB litre^{-1}-infected	**	ns

the sham-injected fish, PCB-exposed fish at both concentrations had lower haematocrits than control; however, differences were significant only between the high PCB and the control. Among the infected groups, the leucocrits of PCB-exposed fish were elevated compared with the control but haematocrits were not different between any exposure concentration. Differences in leucocrits between the two PCB concentrations were not significant in either sham-injected or infected fish.

DMF at 4·3–4·4 μl litre^{-1} had no detectable effect on either leucocrits or haematocrits of rainbow trout at any sampling period (Table 3). Haematocrit values were significantly higher at 34 days than at 7 days ($p < 0.05$), but no significant change with time was shown in the leucocrits.

TABLE 3
LEUCOCRITS AND HAEMATOCRITS OF CONTROL (LAKE WATER) AND 4·3–4·4 μl DMF LITRE^{-1}-EXPOSED RAINBOW TROUT AFTER 7, 14 AND 34 DAYS (DUPLICATES COMBINED)

Exposure length	*Control*		*DMF*	
	Leucocrit (%)	*Haematocrit (%)*	*Leucocrit (%)*	*Haematocrit (%)*
7 days	1·32 ± 0·23 (9)[a]	36·4 ± 4·9 (10)	1·38 ± 0·10 (10)	39·3 ± 2·5 (10)
14 days	1·36 ± 0·28 (9)	40·2 ± 5·1 (10)	1·32 ± 0·16 (9)	40·6 ± 2·8 (10)
34 days	1·23 ± 0·16 (12)	40·8 ± 3·8 (12)	1·28 ± 0·31 (11)	41·0 ± 5·4 (11)

[a] Mean ± standard deviation, ( ) = number of fish.

No adverse effects of copper at 10·9 μg litre^{-1} and 21·5 μg litre^{-1} on rainbow trout were demonstrated, either during the 30-day copper exposure or during the subsequent infection period. Survival and growth were not significantly different at any concentration during the copper exposure ($p = 0.05$). One death occurred during the exposure at 10·9 μg litre^{-1}. The mean weight gain for all fish during this period was 24·6%. The slightly higher mortalities following infection of 40% at 10·9 μg litre^{-1} and 20% at 21·5 μg litre^{-1} were not significant compared with 15% at the control, nor were any differences in time to death observed (Table 4). Haematocrits and leucocrits of trout surviving the 17–18 day infection period showed no significant differences related to copper concentration and few significant bacteria-induced changes (Table 5). Only haematocrits at 10·9 μg litre^{-1} and 21·5 μg litre^{-1} were significantly reduced in infected fish compared with their respective sham-injected groups.

DISCUSSION

This study provided no substantive evidence of altered susceptibility of rainbow trout to *A. hydrophila* following 30 days' continuous exposure to sublethal copper concentrations of 10·9 and 21·5 μg litre^{-1}. Previously published studies have shown increased susceptibility of salmonids to viral and bacterial pathogens following

TABLE 4

MORTALITY AMONG RAINBOW TROUT INFECTED WITH *Aeromonas hydrophila* OR SHAM-INJECTED SUBSEQUENT TO A 30-DAY EXPOSURE TO A CONTROL (LAKE WATER) AND TWO CONCENTRATIONS OF COPPER (REPLICATES COMBINED)

Exposure concentration	*Disease treatment*[a]	*Number/total sample*	*Mortality Percentage*	*Onset (h)*	*Cessation (h)*	*Proportion positive for* A. hydrophila
Control, 2·5 μg Cu litre^{-1}	Sham	0/20	0	—	—	—
Control	Infected	3/20	15	32	192	3/3
10·9 μg Cu litre^{-1}	Sham	0/19	0	—	—	—
10·9 μg Cu litre^{-1}	Infected	8/20[b]	40	32	90	8/8
21·5 μg Cu litre^{-1}	Sham	0/20	0	—	—	—
21·5 μg Cu litre^{-1}	Infected	4/20	20	48	192	4/4

[a] Infected fish were given a 0·1 ml IP injection of an *A. hydrophila* saline suspension containing $1·0 \times 10^9$ CFU per millilitre. Sham-injected fish were given a 0·1 ml IP injection of 0·85% sterile saline.

[b] Not significantly different from control-infected (observed $\chi^2 = 1·96$, degrees of freedom = 1, $0·10 > p < 0·25$).

TABLE 5
LEUCOCRIT AND HAEMATOCRIT VALUES OF RAINBOW TROUT SURVIVING A 30-DAY COPPER EXPOSURE AND SUBSEQUENT *A. hydrophila* INFECTION OR SHAM INJECTION (REPLICATES COMBINED)

Exposure concentration	*Disease treatment*	*Leucocrit (%)*	*Haematocrit (%)*
Control, 2·5 μg Cu litre^{-1}	Sham	1·59 ± 0·31* (19)	30·1 ± 2·9 (20)
Control	Infected	1·72 ± 0·26 (17)	30·5 ± 4·2 (17)
10·9 μg Cu litre^{-1}	Sham	1·85 ± 0·52 (17)	29·2 ± 3·8 (18)
10·9 μg Cu litre^{-1}	Infected	1·53 ± 0·49 (12)	35·9 ± 2·1** (12)
21·5 μg Cu litre^{-1}	Sham	1·63 ± 0·39 (18)	30·6 ± 3·7 (20)
21·5 μg Cu litre^{-1}	Infected	1·42 ± 0·43 (16)	36·2 ± 4·3** (16)

* Mean ± standard deviation, ( ) = number of fish.
** Statistically different from sham at same Cu exposure concentration, $p < 0.05$.

copper exposure (Stevens, 1977; Hetrick *et al.*, 1979; Knittel, 1981). However, Knittel (1981) found that steelhead trout acclimated to copper stress and that their infection susceptibility appeared to be dependent upon copper concentration as well as upon exposure time. His fish at lower concentrations showed increased susceptibility sooner, and also lost this susceptibility sooner, than those exposed at higher concentrations. Hetrick *et al.* (1979) also showed a significant decline in mortality of rainbow trout due to IHN virus between 3 and 7 days' exposure to copper. The absence of detectable changes in haematocrits due to copper exposure in this study was consistent with the results on other freshwater fish exposed to similar copper concentrations (McKim *et al.*, 1970; Christensen *et al.*, 1972). No studies were found that evaluated copper effects on leucocrits or white blood cell counts of fish.

Results of the PCB experiment presented here showed decreased susceptibility of rainbow trout to *A. hydrophila* infection after 30 days of exposure to 7·3 and 14·7 μg litre^{-1} of PCB. In a prior experiment of mine, three to twenty control fish died following *A. hydrophila* inoculation (3×10^7 CFU per fish) while no fish exposed to PCB at 4·0 μg litre^{-1} and 14·5 μg litre^{-1} for 30 days died following the bacterial challenge. In the present study, slight, although statistically significant, increases in mean leucocrit values (15% and 19% higher than controls at 7·3 and 14·7 μg litre^{-1}, respectively) and decreases in mean haematocrit values (10% lower than controls at 14·7 μg litre^{-1}) resulted from PCB exposure. While the mechanisms responsible and the cell types involved (in the case of the leucocrit) are unknown, several interesting speculations can be made from these observations. PCB-induced increases in various types of leucocytes could explain the observed decreased susceptibility (increased resistance) in PCB-exposed fish. Some of these cells are active in the

body's primary inflammatory defence mechanism against pathogens. Nestel & Budd (1975) found significant nephrosis and reduced splenic white pulp in rainbow trout fed 1–100 ppm Aroclor 1254 for 330 days. If similar tissue injury occurred during this exposure, the elevated leucocrits may represent a non-specific inflammatory response to tissue damage or, maybe less likely, a functional overcompensation by an injured spleen or kidney. The decreased haematocrits in PCB-exposed fish could also result from tissue damage or functional impairment of erythropoiesis.

Another possibility is that the highly bioconcentrated PCB molecules may stimulate blastogenesis of lymphocytes, thereby enhancing the fishes' immunological responses. Possibly, the PCB molecule could act as a mitogen, stimulating lymphocytes non-specifically, or it could act as a hapten and elicit a specific immune response. Chilmonczyk (1978) showed that peripheral blood lymphocytes from rainbow trout can be stimulated non-specifically by mitogens. A study by Silkworth & Loose (1979) gives more credence to the theory of stimulation by PCB molecules. Splenic lymphocyte cultures from their mice fed 167 ppm PCB (1016) for 13–41 weeks showed a 56 % enhancement of the mixed lymphocyte culture response and a time-dependent enhancement of up to 64 % above control values following mitogen stimulation.

The increased leucocrits and decreased haematocrits of infected fish compared with their respective sham-injected groups reflected some of the haematological responses of the fish to the bacteria. The increased leucocrits probably represented the inflammatory response against the bacteria (Roberts, 1978) whilst the decreased haematocrits reflected extensive internal haemorrhagic lesions. Lester & Budd (1979) reported reduced haematocrits and elevated leucocyte counts in coho salmon three weeks after *V. anguillarum* inoculation and Barham *et al.* (1980) showed decreased haematocrits in *A. hydrophila*-infected rainbow trout.

Leucocrits and haematocrits appear useful for detecting small, although possibly physiologically significant, changes. However, because of the wide inherent biological variability in these parameters, fairly large numbers of individuals must be sampled to detect these differences statistically. In this study, sampling and measuring three replicate samples per fish (whenever possible) was very time-consuming, particularly because of the standardised sampling intervals. Figure 1 represents an evaluation of replicate sample variability of leucocrits using the data subset with three measurements per fish (representing 170 fish (61 %)). Because no differences in the replicate variability were apparent either between treatments within an experiment or between the experiments, data from the copper and PCB experiments were combined. Comparison of this Figure with standard deviations shown in Tables 2 and 5 shows that replicate sample variability is considerably less than biological variability. It appears that two (or even one) measurements per fish could be acceptable in a large sampling. A similar evaluation of haematocrit values supported this conclusion.

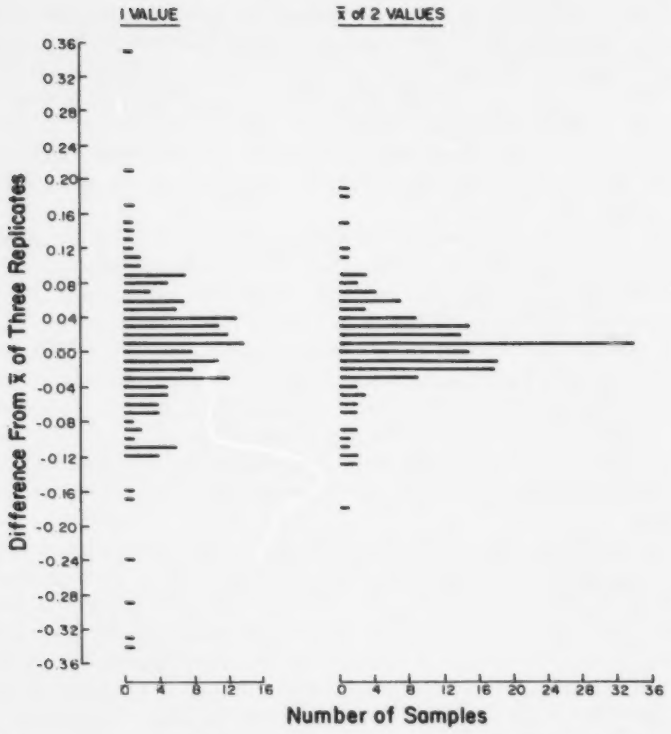

Fig. 1. Precision of leucocrit measurements represented by the difference of the mean of three replicate values from one value and the mean of two values randomly selected from each subset with three replicates. Data from PCB and Cu experiments combined.

The usefulness of leucocrits and haematocrits in evaluating pollutant effects and chemical–disease interactions could be expanded with supportive information from differential cell counts and from functional assays with blood cell cultures.

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